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Plant Gene Register

Chloroplastic Glutamine Synthetase from *Brassica napus*

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In higher plants, the GS/glutamate synthase cycle provides the only efficient pathway for the conversion of inorganic nitrogen to the organic form (Stewart et al., 1980). In this connection, GS catalyzes the ATP-dependent condensation of ammonia with glutamate to yield Gln. Ammonia, the ultimate inorganic form of nitrogen available to the plant, is released by a variety of physiological processes in different organs and subcellular locations within the plant (Miflin and Lea, 1980). Photorespiration in the leaves of C_3 plants represents an important secondary source of ammonia that is reassimilated by GS activity (GS_2) located in the stroma of chloroplasts (Woo et al., 1982; Wallsgrove et al., 1987).

To study the plastidic GS isoenzyme of *Brassica napus*, we screened a λ-ZAPII cDNA library constructed using poly(A)$^+$ mRNA obtained from rape leaves (Table I). The pGS185 cDNA clone from pea (Tingey et al., 1988) was used as a hybridization probe to identify positive phage plaques. Partial sequence analysis and restriction mapping of isolated clones indicated that the coding regions of all cDNAs were identical. The sequence of the longest cDNA, a putative full-length clone of 1575 bp, has been called BnGS18. The nucleotide sequence of this cDNA shows the occurrence of an open reading frame of 1284 nucleotides, a 94-nucleotide 5′ untranslated region, and a 177-nucleotide noncoding 3′ end followed by a polyadenylation stretch of 20 nucleotides.

The open reading frame encodes a precursor protein for a GS_2 subunit of 428 amino acids. If we assume the same processing site for the removal of the transit peptide, as identified by sequence similarity with other plastidic GS enzymes (Lightfoot et al., 1988; Tingey et al., 1988; Peterman and Goodman, 1991; Becker et al., 1992), the molecular mass of the mature protein would be nearly 42 kD. This is in agreement with the apparent molecular mass of the GS_2 subunit as determined by western analysis of rape leaf soluble proteins (data not shown). The amino acid sequence of the mature GS_2 subunit from *B. napus* exhibits 94% identity with the GS_2 sequence of *Arabidopsis thaliana*, another species of Brassicaceae (Peterman and Goodman, 1991). Furthermore, comparison with legume species revealed 88 and 87% similarity with the bean (Lightfoot et al., 1988) and pea sequence (Tingey et al., 1988), respectively.

A comparison of the transit peptide sequences shows less conservation, although there are regions of similarity. The first 147 nucleotides of the open reading frame translate into a polypeptide with a high proportion of basic amino acid residues (18%) that is completely devoid of acidic amino acids; both are characteristics of chloroplast transit peptide sequences (Karlin-Neumann et al., 1986). Further experiments are currently in progress to isolate additional GS-specific transcripts from a root cDNA library of *B. napus*. When this work is complete it will be possible to examine the regulation and expression of GS gene(s) from a nonlegume species.

Table I. *Characteristics of GS cDNA from Brassica napus*

Organism:	*Brassica napus* var Arabella.
Gene Product:	Chloroplastic GS (EC 6.3.1.2).
Techniques:	Screening of λ-ZAPII cDNA library constructed from green leaves using a pea cDNA probe specific for the plastidic isoenzyme (clone pGS 185); double-stranded plasmid (pBluescript) dideoxynucleotide sequencing of both strands by primer jumping.
Method of Identification:	Comparison of the nucleotide sequence and the deduced amino acid sequence with previously described GS sequences from other higher plants.
Features of cDNA:	The clone BnGS18 is 1575 nucleotides in length and consists of a 94-nucleotide 5′ untranslated region, a 1284-nucleotide open reading frame, and a 197-nucleotide 3′ untranslated segment.
Regulation:	Light-stimulated accumulation in green tissue of a 1.7-kb transcript as determined by northern analysis.
Features of Protein:	This cDNA contains an open reading frame of 428 amino acids (M_r 47,342), which encodes a 49-amino transit peptide (M_r 5,382) and a 379-amino acid mature protein (M_r 41,978).
Antibodies:	Polyclonal antibodies raised against GS_2 from *Sinapis alba* cross-react with GS_2 from *Brassica napus*.
Subcellular Location:	Chloroplastic.

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Abbreviations: GS, glutamine synthetase; GS_2, chloroplastic GS.

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